

FORM-PTO-1390 (Rev. 9-2001)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER  003300-908
<b>TRANSMITTAL LETTER TO THE UNITED STATES                  DESIGNATED/ELECTED OFFICE (DO/EO/US)                  CONCERNING A FILING UNDER 35 U.S.C. 371</b>			U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) Unassigned <b>10/069068</b>
INTERNATIONAL APPLICATION NO. PCT/SE00/01776	INTERNATIONAL FILING DATE 14 September 2000	PRIORITY DATE CLAIMED 17 September 1999 28 January 2000	

TITLE OF INVENTION  
**NEW MOLECULARLY IMPRINTED POLYMERS GRAFTED ON SOLID SUPPORTS**

APPLICANT(S) FOR DO/EO/US  
**BÖRJE SELLERGREN, CLAUDIA SULITZKY and BÄRBEL RÜCKERT**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:  
 It is contemplated that this application be prosecuted while using Claims 1 to 23 that were submitted on March 22, 2001 as further amended in the Preliminary Amendment filed herewith.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
  - b. ☒ has been communicated by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is attached hereto.
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ have been communicated by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

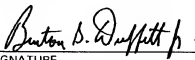
Items 11 to 20 below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:

Certified copies of Swedish Application No. 99033870-0, filed 17 September 1999, and Swedish Application No. 0000294-9, filed 28 January 2000, were submitted during the international phase of prosecution. Thus, the claims for priority have been perfected.



**21839**

U.S. APPLICATION NO. (if known, see 37 CFR 1.51) <b>unassigned 10/069068</b>		INTERNATIONAL APPLICATION NO. <b>PCT/SE00/01776</b>		ATTORNEY'S DOCKET NUMBER <b>003300-908</b>	
21. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS	PTO USE ONLY
<b>Basic National Fee (37 CFR 1.492(a)(1)-(5)):</b> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to U.S. PATENT AND TRADEMARK OFFICE and International Search Report not prepared by the EPO or JPO ..... \$1,040.00 (960) International preliminary examination fee (37 CFR 1.482) not paid to U.S. PATENT AND TRADEMARK OFFICE but International Search Report prepared by the EPO or JPO ..... \$890.00 (970) International preliminary examination fee (37 CFR 1.482) not paid to U.S. PATENT AND TRADEMARK OFFICE but international search fee (37 CFR 1.445(a)(2)) paid to U.S. PATENT AND TRADEMARK OFFICE ..... \$740.00 (958) International preliminary examination fee (37 CFR 1.482) paid to U.S. PATENT AND TRADEMARK OFFICE but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$710.00 (956) International preliminary examination fee (37 CFR 1.482) paid to U.S. PATENT AND TRADEMARK OFFICE and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$100.00 (962)					
<b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>\$ 1,040.00</b>	
Surcharge of \$130.00 (154) for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)). 20 <input type="checkbox"/> 30 <input type="checkbox"/>				<b>\$</b>	
Claims	Number Filed	Number Extra	Rate		
Total Claims	36 -20 =	16	X\$18.00 (966)	\$ 288.00	
Independent Claims	2 -3 =	0	X\$84.00 (964)	\$ --	
Multiple dependent claim(s) (if applicable)			+ \$280.00 (968)	\$ --	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				<b>\$ 1,328.00</b>	
Reduction for 1/2 for filing by small entity, if applicable (see below).				+ \$ 664.00	
<b>SUBTOTAL =</b>				<b>\$ 664.00</b>	
Processing fee of \$130.00 (156) for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(i)). 20 <input type="checkbox"/> 30 <input type="checkbox"/>				<b>\$ --</b>	
<b>TOTAL NATIONAL FEE =</b>				<b>\$ 664.00</b>	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property				+ \$ 40.00	
<b>TOTAL FEES ENCLOSED =</b>				<b>\$ 704.00</b>	
				Amount to be refunded: \$	
				charged: \$	
a. <input checked="" type="checkbox"/> Small entity status is hereby claimed. b. <input checked="" type="checkbox"/> A check in the amount of \$ <u>704.00</u> to cover the above fees is enclosed. c. <input type="checkbox"/> Please charge my Deposit Account No. <u>02-4800</u> in the amount of \$_____ to cover the above fees. A duplicate copy of this sheet is enclosed. d. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4800</u> . A duplicate copy of this sheet is enclosed. <b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO: Benton S. Duffett, Jr. BURNS, DOANE, SWECKER & MATHIS, L.L.P. P. O. Box 1404 Alexandria, Virginia 22313-1404 (703) 836-6620					
				SIGNATURE  Benton S. Duffett, Jr. NAME	
				22,030 REGISTRATION NUMBER	February 21, 2002 DATE

Patent  
Attorney's Docket No. 003300-908

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of	)	
	)	
BÖRJE SELLERGREN et al.	)	BOX PCT
	)	
Application No.: (unassigned)	)	Attention: DO/EO/US
	)	
Filed: February 21, 2002	)	Group Art Unit: (unassigned)
	)	
For: NEW MOLECULARLY IMPRINTED )	)	Examiner: (unassigned)
POLYMERS GRAFTED ON SOLID )	)	
SUPPORTS	)	

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

This is a national phase filing of International Application No. PCT/SE00/01776,  
filed September 14, 2000.

It is contemplated that this Application be prosecuted while using Claims 1 to 23  
that were submitted on March 22, 2001 during the international phase of prosecution as  
further amended herein.

**IN THE ABSTRACT:**

Please add the Abstract of the Disclosure that is provided on a separate sheet.

**IN THE CLAIMS:**

Kindly replace Claims 4, 5, 7, 10, 11, 21 and 23 as follows:

4. (Amended) A supported molecularly imprinted polymer according to claim 1, wherein the support is selected from the group consisting of porous and non-porous, planar and non-planar inorganic and organic supports.

5. (Amended) A supported molecularly imprinted polymer according to claim 1, wherein the support is a particle and the free radical initiator is an azo-initiator that is bound to the surface of the particle.

7. (Amended) A supported molecularly imprinted polymer according to claim 1, wherein the initiator is an azo-bis-amidine initiator that is adsorbed to the surface of the support and is insoluble in the polymerisation medium.

10. (Amended) A supported molecularly imprinted polymer according to claim 1, wherein the polymerisation on the support is repeated at least once with a different composition to obtain at least one further layer of a molecularly imprinted polymer; a layer of different polarity; or a layer of other functional properties.

11. (Amended) A supported molecularly imprinted polymer according to claim 1, wherein the template is selected from the group consisting of organic or inorganic molecule entities, ions, antibodies, antigens, amino acids, peptides, proteins, nucleotides, DNA-bases, carbohydrates, drugs, pesticides, and derivatives thereof.

21. (Amended) A method according to claim 12, wherein the polymerisation on the support is repeated at least once with a different composition to obtain at least one further layer of a molecularly imprinted polymer; a layer of different polarity; or a layer of other functional properties.

23. (Amended) Azoinitiator as a means of carrying out the method of claim 12, characterised in that it is the reaction product of glycidoxypolytrimethoxysilane (GPS) and azo-bis-(cyanopentanoic acid) (ACPA).

Please cancel Claim 22 without prejudice or disclaimer.

Please add the following new Claims 24 to 37:

24. (New) A supported molecularly imprinted polymer according to claim 2, wherein the support is selected from the group consisting of porous and non-porous, planar and non-planar inorganic and organic supports.

25. (New) A supported molecularly imprinted polymer according to claim 3 wherein the support is selected from the group consisting of porous and non-porous, planar and non-planar inorganic and organic supports.

26. (New) A supported molecularly imprinted polymer according to claim 2, wherein the support is a particle and the free radical initiator is an azo-initiator that is bound to the surface of the particle.

27. (New) A supported molecularly imprinted polymer according to claim 2, wherein the initiator is an azo-bis-amidine initiator that is adsorbed to the surface of the support and is insoluble in the polymerisation medium.

28. (New) A supported molecularly imprinted polymer according to claim 2, wherein the polymerisation on the support is repeated at least once with a different composition to obtain at least one further layer of a molecularly imprinted polymer; a layer of different polarity; or a layer of other functional properties.

29. (New) A supported molecularly imprinted polymer according to claim 2, wherein the template is selected from the group consisting of organic or inorganic molecule entities, ions, antibodies, antigens, amino acids, peptides, proteins, nucleotides, DNA-bases, carbohydrates, drugs, pesticides, and derivatives thereof.

30. (New) A method according to claim 13, wherein the polymerisation on the support is repeated at least once with a different composition to obtain at least one further layer of a molecularly imprinted polymer; a layer of different polarity; or a layer of other functional properties.

31. (New) Azoinitiator as a means of carrying out the method of claim 13, characterised in that it is the reaction product of glycidoxypolytrimethoxysilane (GPS) and azo-bis-(cyanopentanoic acid) (ACPA).

32. (New) A chromatography process wherein the supported molecularly imprinted polymer of claim 1 is utilized.

33. (New) A separation process wherein the supported molecularly imprinted polymer of claim 1 is utilized.

34. (New) A chemical sensor comprising the supported molecularly imprinted polymer of claim 1.

35. (New) A process for molecular recognition as stationary phase in capillaries wherein the supported molecularly imprinted polymer of claim 1 is utilized.

36. (New) A process for selective sample enrichment wherein the supported molecularly imprinted polymer of claim 1 is utilized.

37. (New) A catalysis process wherein the molecularly imprinted polymer of claim 1 is utilized.

Application No. (unassigned)  
Attorney's Docket No. 003300-908  
Page 6

**REMARKS**

The present Amendment modifies the claim format and eliminates the use of multiple dependency only. The subject matter of Claim 22 is now presented in new claims 32 to 37.

An Information Disclosure Statement is being filed herewith.

The examination and allowance of the Application are respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: 

Benton S. Duffett, Jr.  
Registration No. 22,030

P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

Filed: February 21, 2002



Application No. (Unassigned)  
Attorney's Docket No. 003300-908  
Abstract - Page 1 of 1

**Attachment to Preliminary Amendment dated February 21, 2002**

**ABSTRACT OF THE DISCLOSURE**

The invention refers to a molecularly imprinted polymer, a method of preparing a molecularly imprinted polymer material, and the use thereof. According to the invention a support and a composition comprising at least one monomer, and a template, in a polymerisation medium is polymerized with a free radical initiator, whereafter the template is removed from the molecularly imprinted polymer obtained. The polymerisation is confined to the surface of the support, preferably by confining the free radical initiator to the support by bonding or adsorption. The molecularly imprinted polymer may be used in chromatography, for separations, in chemical sensors, in molecular recognition as stationary phase in capillaries, in selected sample enrichment or in catalysis.

Application No. Unassigned  
Attorney's Docket No. 003300-908  
Mark-up of Claims - Page 1

**Attachment to Preliminary Amendment dated February 21, 2002**  
**Mark-Up of Claims 4, 5, 7, 10, 11, 21, and 23**

4. (Amended) A supported molecularly imprinted polymer according to [any one of claims 1-3] claim 1, wherein the support is selected from the group consisting of porous and non-porous, planar and non-lanar inorganic and organic supports.
5. (Amended) A supported molecularly imprinted polymer according to [any one of claims 1-4] claim 1, wherein the support is a particle and the free radical initiator is an azo-initiator that is bound to the surface of the particle.
7. (Amended) A supported molecularly imprinted polymer according to [any one of claims 1-4] claim 1, wherein the initiator is an azo-bis-amidine initiator that is adsorbed to the surface of the support and is insoluble in the polymerisation medium.
10. (Amended) A supported molecularly imprinted polymer according to [any one of claims 1-9] claim 1, wherein the polymerisation on the support is repeated at least once with a different composition to obtain at least one further layer of a molecularly imprinted polymer; a layer of different polarity; or a layer of other functional properties.

Application No. Unassigned  
Attorney's Docket No. 003300-908  
Mark-up of Claims - Page 2

**Attachment to Preliminary Amendment dated February 21, 2002**  
**Mark-Up of Claims 4, 5, 7, 10, 11, 21, and 23**

11. (Amended) A supported molecularly imprinted polymer according to [any one of claim 1-10] claim 1, wherein the template is selected from the group consisting of organic or inorganic molecule entities, ions, antibodies, antigens, amino acids, peptides, proteins, nucleotides, DNA-bases, carbohydrates, drugs, pesticides, and derivatives thereof.

21. (Amended) A method according to [any one of claims 12-20] claim 12, wherein the polymerisation on the support is repeated at least once with a different composition to obtain at least one further layer of a molecularly imprinted polymer; a layer of different polarity; or a layer of other functional properties.

23. (Amended) Azoinitiator as a means of carrying out the method of [any one of claims 12-21] claim 12, characterised in that it is the reaction product of glycidoxypolytrimethoxysilane (GPS) and azo-bis-(cyanopentanoic acid) (ACPA).

5/pst

1

NEW MOLECULARLY IMPRINTED POLYMERS GRAFTED ON SOLID  
SUPPORTS

Technical Field of the Invention

The present invention relates to a molecularly imprinted polymer, to a method for preparing said molecularly imprinted polymer, and to the use of said molecularly imprinted polymer.

Background Art

In the fields of medical, dietary, environmental and chemical sciences there is an increasing need for the selective separation of specific substances in complex mixtures of related substances. The end goal can be the preparative isolation of a certain compound or compounds or measurements of their concentration. Molecularly imprinted polymers (MIPs) often exhibit a high selectivity towards their substrate in analogy with the antibody-antigen complementarity. (1, 2) The technique shows promise in chiral separations of for example amino acid derivatives, peptides, phosphonates, aminoalcohols and beta-blocking compounds, affinity chromatography of nucleotides and the DNA-bases as well as substitute for antibodies in immunoassays for commercial drugs. Molecular imprinting (MI) consists of the following key steps: (1) Functional monomers are allowed to interact reversibly with a template molecule in solution. (2) The hereby formed template assemblies are copolymerised with a cross-linking monomer resulting in a cross-linked network polymer. (3) The template is displaced and the resulting MIP material can be used for selective molecular recognition of the corresponding compound. If the MIP material is crushed and sieved it can be packed in a chromatographic column and used for chromatographic separation of the template from structurally related analogs. Analytical as well as preparative applications are here possible. Preparative applications can be separation

of a compound from a complex mixture of structurally related compounds and isolation of the compound. This can be through an affinity chromatographic procedure where pH, ion strength or solvent gradients can be used in order to control the strength of interaction with the stationary phase. The separation can target enantiomers or diastereomers in a mixture of enantiomers or diastereomers of one or many compounds. Analytical applications can in addition to the above mentioned separations be: competitive binding assays, chemical sensors or selective sample enrichments.

Currently the most widely applied technique to generate molecularly imprinted binding sites is represented by the non-covalent route developed by the group of Mosbach(3). This makes use of non-covalent self-assembly of the template with functional monomers prior to polymerisation, free radical polymerisation with a cross-linking monomer and then template extraction followed by re-binding by non-covalent interactions. Although the preparation of a MIP by this method is technically simple it relies on the success of stabilisation of the relatively weak interactions between the template and the functional monomers. Stable monomer-template assemblies will in turn lead to a larger concentration of high affinity binding sites in the resulting polymer. The materials can be synthesized in any standard equipped laboratory in a relatively short time and some of the MIPs exhibit binding affinities and selectivities in the order of those exhibited by antibodies towards their antigens. Most MIPs are synthesized by free radical polymerisation of functional monounsaturated (vinyllic, acrylic, methacrylic) monomers and an excess of cross-linking di- or triunsaturated (vinyllic, acrylic, methacrylic) monomers resulting in porous organic network materials. These polymerisations have the advantage of being relatively robust allowing polymers to be prepared in high yield using different solvents (aqueous or organic) and at different

temperatures (4). This is necessary in view of the varying solubilities of the template molecules.

The most successful non-covalent imprinting systems are based on commodity acrylic or methacrylic monomers, such as methacrylic acid (MAA), cross-linked with ethyleneglycol dimethacrylate (EDMA). Initially, derivatives of amino acid enantiomers were used as templates for the preparation of imprinted stationary phases for chiral separations (MICSPs) but this system has proven generally applicable to the imprinting of templates allowing hydrogen bonding or electrostatic interactions to develop with MAA. (5, 6) The procedure applied to the imprinting with L-phenylalanine anilide (L-PA) is outlined in Fig. 1. In the first step, the template (L-PA), the functional monomer (MAA) and the cross-linking monomer (EDMA) are dissolved in a poorly hydrogen bonding solvent (diluent) of low to medium polarity. The free radical polymerisation is then initiated with an azo initiator, commonly azo-N,N'-bis-isobutyronitrile (AIBN) either by photochemical homolysis below room temperature (6, 7) or thermochemically at 60°C or higher (5). Lower thermochemical initiation temperatures down to 40°C or 30°C may be obtained using azo-N,N'-bis-divaleronitrile (ABDV) and V70 resp. instead of AIBN as initiator (see). (7, 8) In the final step, the resultant polymer is crushed by mortar and pestle or in a ball mill, extracted by a Soxhlet apparatus, and sieved to a particle size suitable for chromatographic (25-38  $\mu\text{m}$ ) or batch (150-250  $\mu\text{m}$ ) applications. (6) The polymers are then evaluated as stationary phases in chromatography by comparing the retention time or capacity factor ( $k'$ ) (9) of the template with that of structurally related analogs.

As appears from above MIPs have so far been prepared in the form of continuous blocks that need to be crushed and sieved before use. This results in a low yield of irregular particles, a high consumption of template and a material exhibiting low chromatographic efficiency. There

is therefore a need for MI-materials that can be prepared in high yield in the form of regularly shaped particles with low size dispersity and a controlled porosity. These are expected to be superior in terms of mass transfer characteristics and sample load capacity compared to the materials obtained from the monolithic approach.

Such MIPs have been previously prepared through suspension(10, 11)- polymerisation techniques, dispersion polymerisation(12) or precipitation polymerisation(13). This resulted in spherical particles of a narrow size distribution. These procedures have the limitation of being very sensitive to small changes in the manufacturing conditions and the type of solvents and polymerisation conditions that can be applied. Thus the procedures need careful optimization for each new template target which significantly reduces the usefulness of this route. Moreover conditions leading to low dispersity spherical particles may not be compatible with conditions leading to high selectivity and affinity for the template target.

An alternative to this procedure is the coating of preformed support materials.(14-16) MIPs have been prepared as grafted coatings on oxide supports(14, 16) on organic polymer supports(15) and on the walls of fused silica capillaries(17-19). The former technique allows the use of the wide variety of oxide support materials available with different sizes and porosities. Grafting techniques to prepare organic polymer coatings are expected to be generally applicable to molecular imprinting since the structure of the underlying support is already fixed. Thus compared to the large number of factors influencing the end result in suspension or precipitation type polymerisations a smaller number of factors is likely to influence the end result in the preparation of the imprinted coatings. This will make the grafted coatings techniques less sensitive to changes in conditions offering a more robust method. These types of coating techniques are furthermore applicable to modify surfaces of

monolithic type supports or microchips prepared by lithographic techniques. The oxide based materials are rigid porous supports with a limited inner pore volume. An alternative support that could potentially carry more grafted imprinted polymer per unit weight and thus allow a higher density of imprinted sites would be to make use of swellable organic resins. In this context Metrifiield resins containing grafted initiator or monomer could be used.

5       Sofar most imprinted coatings have been prepared by grafting polymers to the various surfaces. Thus the surface contains prior to polymerisation polymerizable double bonds that can add to the growing polymer chains in solution linking them to the surface. The problem with this technique is the presence of initiator in solution requiring the monomer mixture to be applied as a liquid thin film on the surface prior to polymerisation. Thus the exact amount of monomers that will coat the available surface with an up to ca 100 Å thick liquid film is dissolved together with initiator in an excess of solvent. Thereafter the modified support is added and the solvent evaporated to leave the monomer film and initiator on the surface. Polymerisation is then carried out usually at elevated temperatures. With this procedure the thickness of the polymer layer is difficult to control and capillary forces upon evaporation of solvent may cause incomplete wetting of the surface. Moreover a continuous method of synthesising the particles is difficult to envisage with this method.

10       A considerable improvement in this regard would be to confine the initiator radicals to the support surface (Fig. 2). (20, 21) In absence of chain transfer this would lead to chain growth occurring only from the surface of the support with no polymerisation occurring in solution. For molecular imprinting this would have important consequences. For instance the polymerisation can be carried out on the surface of initiator modified support partic-



es suspended in a mixture of the monomers and solvent. This would allow polymerisation in a simple tank reactor by either thermal or photochemical initiation. The latter technique would allow the particles to be modified during the sedimentation possibly leading to a continuous method for preparing the imprinted composite particles (Fig. 3). Polymerisation would here only occur on the particle surface leaving the solution containing the monomers unreacted. The monomer solution can thus be reused for the coating of several batches of particles. The problem of confining polymer chain growth to the support surface and suppress it in solution can be solved by attaching the radical initiator so that the radical formed upon bond homolysis remains bound to the surface. Alternatively the radical formed that is not attached to the surface should undergo rapid reaction to give an unreactive species. It should be possible to prepare the grafted coatings using monomers such as those based on styren/divinylbenzene, methacrylates, acrylates, acrylamides and in the presence of one or more template molecules.

#### Summary of the Invention

Thus, the present invention relates to a molecularly imprinted polymer obtainable by polymerising a composition comprising at least one monomer, and a template, on a support in a polymerisation medium with a free radical initiator, whereafter the template is removed from the molecularly imprinted polymer obtained, said polymerisation being confined to the surface of the support.

The invention further relates to a method for preparing a molecularly imprinted polymer which comprises polymerising a composition comprising at least one monomer, and a template, on a support in a polymerisation medium with a free radical initiator, whereafter the template is removed from the molecularly imprinted polymer obtained, said polymerisation being confined to the surface of the support.

Still further the invention relates to the use of a molecularly imprinted polymer as defined above in chromatography, for separations, in chemical sensors, in molecular recognition as stationary phase in capillaries, in selective enrichment or in catalysis.

These and other advantages and characterising features of the present invention will appear from the following specification and the appended claims.

#### Brief Description of the Drawings

Fig. 1 illustrates molecular imprinting with L-phenylalanine anilide (L-PA).

Fig. 2 illustrates the procedure of confining initiator radicals to the surface of a support.

Fig. 3 illustrates a method for preparing imprinted composite particles.

Fig. 4A illustrates the use of a presynthesized azosilane initiator where both ends may be attached to the surface of a support.

Fig. 4B illustrates an initiator that may be preadsorbed on a support surface and that is insoluble in the monomer containing solution.

Fig. 4C illustrates the use of microwaves to selectively heat the particle surface.

Fig. 4D illustrates the use of iniferters such as dithiocarbamate coupled onto the surface.

#### Detailed Description of the Invention

The invention will now be described in more detail with reference to a number of non-limiting examples:

The invention refers to a material that consists in a support (porous or nonporous material or planar surface) coated with a polymer layer, a method for its fabrication and use of said material in for instance chromatography, for separations, in chemical sensors, in selective sample enrichment, in molecular recognition as stationary phase in capillaries or in catalysis. The material is prepared by grafting a polymer layer on the surface of a preformed organic or inorganic support material or surface. The grafting can be combined with the technique of molecular imprinting.

In one embodiment of the present invention the polymerisation is confined to the surface of the support by confining the free radical initiator to the support. According to one aspect the free radical initiator is bound (covalently or non-covalently such as e.g. by hydrogen bonds) to the surface of the support. According to another aspect the free radical initiator is adsorbed to the surface of the support, preferably by dissolving it in a solvent for the free radical initiator, applying the solution to the support, and removing the solvent, said free radical initiator being insoluble in the polymerisation medium or remaining attached to the support surface by adsorptive forces.

In another embodiment of the present invention the polymerisation is confined to the surface of the support by subjecting the composition, the support and the free radical initiator to microwave irradiation which selectively heats the support and thereby initiates a polymerisation reaction at the surface of the support.

In a further embodiment of the present invention the polymerisation is repeated at least once with a different composition to obtain at least one further layer of molecularly imprinted polymer. This allows the manufacturing of layered surfaces containing one or more imprinted layers using possibly different templates and layers of different polarity or other functional properties.

The support used in the present invention is preferably selected from the group consisting of porous and non-porous, planar and non-planar inorganic and organic supports. As examples of such support materials may be mentioned oxides such as alumina and silica, and organic resins in the form of particles such as spheres, or sheets.

The template used in the present invention may be any molecule or ion and is preferably selected from the group consisting of organic or inorganic molecule entities, ions, antibodies, antigens, amino acids, peptides, proteins, nucleotides, DNA-bases, carbohydrates, drugs, pesticides, and derivatives thereof, etc.

The expression "polymerisation medium" as used herein means a liquid medium in which the polymerisation is carried out. The polymerisation medium may e.g. be a solvent in which the monomers are soluble. It may also be a monomer acting as a solvent for the other components of the polymerisable composition.

The support surface is prepared as follows. A free radical initiator is bound to the surface either covalently or noncovalently so that the free radicals generated upon initiation remain confined to the surface or vicinity of the surface. The absence of polymer propagation in solution will lead to a higher accessibility of the monomers at the surface. Furthermore this method will allow the tuning of the thickness of the polymer layer.

Surface attachment of a free radical initiator has been disclosed generally by Guyot et.al. (21) and Tsubokawa et.al. (22, 23). It relies on presilanization of the surface using 3-aminopropyltriethoxysilane or a glycidoxypropylsilane (GPS) followed by reaction of the amino groups or the epoxy groups with an azoinitiator such as azo-bis(cyanopentanoic acid, ACPA) leading to the formation of an amide (using DCC as condensing reagent) or ester link between the surface and the azoinitiator. Also peroxy initiators may be used although better re-

sults are obtained using the grafted azoinitiator followed by photochemical initiation. High yields of grafted polymer are obtained using silica reacted with toluene-2,4-diisocyanate (TDI) followed by reaction with ACPA.

5 Example 1

Coupling of initiator to amino, epoxy or chloromethyl modified supports or resins

Epoxy and chloromethyl modified supports: A typical example is as follows. Into a flask, 3 g of epoxy modified particles 50 mL of DMSO, 0.5 g of ACPA and picoline  
10 were charged. The reaction mixture was stirred for 5 h at 50°C. After the reaction the particles were washed with methanol and dried.

Amino modified supports: A typical example is as  
15 follows. Into a flask, 3 g of epoxy modified particles 50 mL of DMF, 0.5 g of ACPA and dicyclohexyldicarbodiimide (DCCI) and base were charged. The reaction mixture was stirred for 5 h. After the reaction the particles were washed with methanol and dried.

20 The above procedure does not confine all initiator radicals to the surface since the initiator is bound at only one position. This invention describes three alternative procedures to confine the polymerisation to the surface.

25 1. The use of a presynthesized azosilane (Fig. 4A). This will more likely lead to a two point attachment of the initiator to the surface.

Example 2

Synthesis of azosilane for two point coupling of an azo-  
30 initiator to a surface or support

The azosilane was synthesized by mixing 0.5 mole glycidoxypolypropyltrimethoxysilane (GPS) and 0.25 mole ACPA in 200 mL isopropanol and catalytic amounts of picoline. The reaction was allowed to continue at room temperature  
35 and the product isolated by evaporation to dryness followed by purification by column chromatography giving the product in 60 % yield.

Example 3

Coupling of silane to a surface

- 5       The silane was coupled by reaction in water at low temperature (20°C) for 24 hours.

2.       Preadsorption of an initiator that is insoluble in the monomer containing solution. Thus, a polar water soluble initiator as for instance an azo-bis-amidine, (24) 10 can be adsorbed to the surface from aqueous solvent, the surface dried and then the polymerisation initiated as described above (Fig. 4B). The free radicals generated from the initiator will stay associated to the surface due to their insolubility in the monomer mixture.

15       Example 4

Adsorption of amidineazoinitiator to a support surface

- An amidineazoinitiator such as 2,2'-azobis(N,N'-dimethyleneisobutyramidine) or 2,2'-azobis(2-amidino- 20 propane) is dissolved in methanol/water and support particles such as silica are added. After several hours of equilibration the solvent is removed by filtration and the particles dried under vacuum.

3.       Use of microwaves to selectively heat the particle surface (Fig. 4C).

25       Example 5

Microwave initiated polymerisation

- Particles are added to a solution of monomers and initiator in a suitable solvent. The polymerisation is initiated by microwave irradiation at a wavelength causing 30 local heating of the particles only.

4.       Use of iniferters such as dithiocarbamate coupled onto the surface (Fig. 4D). (25) (The term "iniferter" is an abbreviation for "initiator + transfer agent + terminator").

Example 6

Synthesis of support or polymeresin bound initiator

- To a surface or polymer containing bound chloromethyl groups is given N,N-diethyldithiocarbamate in solution and the reaction allowed to proceed at elevated temperatures.

Example 7

Synthesis of block-graft imprinted copolymer

- Particles or a surface containing bound dithiocarbamate groups are/is added to a mixture of monomers (concentration about 5 moles/litre), template and solvent under nitrogen. The polymerisation was initiated by irradiation with an ultrahigh pressure mercury UV lamp and allowed to proceed for a certain time. Then the unreacted monomers and template were washed away. The obtained particles or surface can then be immersed in another solution containing another monomer and the procedure repeated. This allows the manufacturing of layered surfaces containing one or more imprinted layers using possibly different templates and layers of different polarity or other functional properties.

Example 8

Endcapping of unreacted silanol groups

- Prior to polymerisation endcapping of unreacted silanol groups can be done. Hexamethylsilazane is here effective. Good wetting is critical for the formation of a homogenous layer fully covering the support. Another possibility to enhance the wetting is to use organosilanes containing functionalities resembling solvents known to be good solvents for the methacrylate polymerisations. Among these chlorinated hydrocarbons are particularly useful.

Grafting of polymer layer

- The polymerisation can be carried out in a stirred suspension of the particles in the monomer mixture since growth only takes place on the surface (see Fig. 3). Thus the initiator modified particles are added to a

monomer containing solution and solvent and possibly a template and the suspension stirred. The polymerisation is then carried out photochemically or thermally. The particles can be based on any inorganic or organic support material and the template on any molecule or ion dissolved in the monomer mixture solution. The grafting can also occur on other surfaces such as those generated by lithographic processes or on the walls of capillaries or fibres. The thickness of the polymer layer is tunable by varying the time of reaction.

#### Example

To a stirred solution of 38 ml (0.2 mole) EDMA, 3.4 ml (40 mmole) MAA and 10 mmole terbutylazine (or no template) in 56 ml dichloromethane is added 5 g of any of the initiator modified particles described in Examples 1-6. The suspension is sparged with nitrogen and the polymerisation initiated by UV irradiation using a standard high pressure mercury lamp at 15°C or by heating to a temperature providing a suitable rate of polymerisation. The suspension is stirred under nitrogen and UV irradiation or heating for 24 h and the particles then filtered, washed and dried under vacuum. The monomer mixture is then used to modify a second batch of particles.

The resulting particles exhibit high selectivity and affinity for the template, terbutylazine.



## LITERATURE

1. Wulff, G. 1995. *Angew. Chem., Int. Ed. Engl.* 34: 1812-32
- 5 2. Bartsch, R. A., Maeda, M. 1998. In *ACS Symposium Series 703*. Washington: Oxford University Press
3. Mosbach, K. 1994. *Trends Biochem. Sci.* 19: 9-14
4. Cowie, J. M. G. 1991. *Polymers: Chemistry & Physics of modern materials*. Glasgow: Blackie and Son
- 10 Ltd.
5. Sellergren, B., Lepistoe, M., Mosbach, K. 1988. *J. Am. Chem. Soc.* 110: 5853-60
6. Sellergren, B., Shea, K. J. 1993. *J. Chromatogr.* 635: 31
- 15 7. O'Shannessy, D. J., Ekberg, B., Mosbach, K. 1989. *Anal. Biochem.* 177: 144-9
8. Sellergren, B. 1989. *Makromol. Chem.* 190: 2703-11
9. Snyder, L. R., Kirkland, J. J. 1979.
- 20 *Introduction to Modern Liquid Chromatography*. US: Wiley
10. Mayes, A. G., Mosbach, K. 1996. *Anal. Chem.* 68: 3769-3774
11. Matsui, J., Okada, M., Tsuruoka, M., Takeuchi, T. 1997. *Anal. Commun.* 34: 85-87
- 25 12. Sellergren, B. 1994. *J. Chromatogr. A.* 673: 133-141
13. Lei, Y., Cormack, P. A. G., Mosbach, K. 1999. *Anal. Commun.* 36: 35-38
14. Wulff, G., Oberkobusch, D., Minarik, M. 1985.
- 30 *React. Polym., Ion Exch., Sorbents.* 3: 261-75
15. Glad, M., Reinholdsson, P., Mosbach, K. 1995. *React. Polym.* 25: 47-54
16. Arnold, F. H., Plunkett, S., Dhal, P. K., Vidyasankar, S. 1995. *Polym. Prepr.* 36(1): 97-8
- 35 17. Schweitz, L., Andersson, L. I., Nilsson, S. 1997. *Anal. Chem.* 69: 1179-1183

18. Brüggemann, O., Freitag, R., Whitcombe, M. J., Vulfson, E. N. 1997. *J. Chromatogr.* 781: 43-53
19. Lin, J.-M., Nakagama, T., Uchiyama, K., Hobo, T. 1997. *J. Liq. Chromatogr. Relat. Technol.* 20: 1489-1506
20. Carlier, E., Guyot, A., Revillon, A. 1991. *React. Polym.* 16: 115
21. Carlier, E., Guyot, A., Revillon, A. 1992. *React. Polym.* 18: 167
22. Tsubokawa, N., Kogure, A., Maruyama, K., Sone, Y., Shimomura, M. 1990. *Polym. J.* 22: 827-833
23. Tsubokawa, N., Shirai, Y., Tsuchida, H., Handa, S. 1994. *J. Polym. Sci. A: Polym. Chem.* 32: 2327-2332
24. Dekking, H. G. G., 1965, *J. Appl. Polym. Sci.* 9, 1641-1651
25. Nakayama, Y.; Matsuda, T. 1999, *Langmuir*, 15, 5560-5566.

CLAIMS

1. A supported molecularly imprinted polymer,  
c h a r a c t e r i s e d in that it is obtainable by

(a) providing a composition comprising a poly-  
merisation medium with at least one functional monomer, a  
5 template, a support, and a free radical initiator;

(b) polymerisation of the composition while con-  
fining the polymerisation to the surface of the support,  
thereby providing a molecularly imprinted polymer on the  
support;

10 (c) separation of the supported molecularly im-  
printed polymer from the polymerisation medium;

(d) removal of the template from the supported  
molecularly imprinted polymer;

(e) reuse of the polymerisation medium for pre-  
15 paring further supported molecularly imprinted polymer by  
repeating steps (a)-(d).

2. A supported molecularly imprinted polymer  
according to claim 1, wherein the polymerisation is con-  
fined to the surface of the support by confining the free  
20 radical initiator to the support.

3. A supported molecularly imprinted polymer  
according to claim 2, wherein the free radical initiator  
is bound or adsorbed to the surface of the support.

4. A supported molecularly imprinted polymer  
25 according to any one of claims 1-3, wherein the support  
is selected from the group consisting of porous and non-  
porous, planar and non-planar inorganic and organic  
supports.

5. A supported molecularly imprinted polymer  
30 according to any one of claims 1-4, wherein the support  
is a particle and the free radical initiator is an azo-  
initiator that is bound to the surface of the particle.

8 A74.34

6. A supported molecularly imprinted polymer according to claim 5, wherein the azoinitiator is bound to the surface of the particle by a two point attachment.

7. A supported molecularly imprinted polymer  
5 according to any one of claims 1-4, wherein the initiator is an azo-bis-amidine initiator that is adsorbed to the surface of the support and is insoluble in the polymerisation medium.

8. A supported molecularly imprinted polymer  
10 according to claim 7, wherein the initiator is 2,2'-azo-bis(2-amidinopropane) or 2,2'-azobis(N,N'-dimethylene-isobutyramidine).

9. A supported molecularly imprinted polymer according to claim 1, wherein the polymerisation is  
15 confined to the surface of the support by subjecting the composition to microwave irradiation which selectively heats the support and thereby initiates a polymerisation reaction at the surface of the support.

10. A supported molecularly imprinted polymer  
20 according to any one of claims 1-9, wherein the polymerisation on the support is repeated at least once with a different composition to obtain at least one further layer of a molecularly imprinted polymer; a layer of different polarity; or a layer of other functional  
25 properties.

11. A supported molecularly imprinted polymer according to any one of claims 1-10, wherein the template is selected from the group consisting of organic or inorganic molecule entities, ions, antibodies, antigens,  
30 amino acids, peptides, proteins, nucleotides, DNA-bases, carbohydrates, drugs, pesticides, and derivatives thereof.

12. A method for preparing a supported molecularly imprinted polymer, characterised by

35 (a) providing a composition comprising a polymerisation medium with at least one functional monomer, a template, a support, and a free radical initiator;

22 -03- 2001

3

(b) polymerising the composition while confining the polymerisation to the surface of the support, thereby providing a molecularly imprinted polymer on the support;

(c) separating the supported molecularly imprinted  
5 polymer from the polymerisation medium;

(d) removing the template from the supported molecularly imprinted polymer;

(e) reusing the polymerisation medium for preparing further supported molecularly imprinted polymer by  
10 repeating steps (a)-(d).

13. A method according to claim 12, wherein the polymerisation is confined to the surface of the support by confining the free radical initiator to the support.

14. A method according to claim 13, wherein the free  
15 radical initiator is bound or adsorbed to the surface of the support.

15. A method according to claim 14, wherein the support is a particle and the initiator is an azo-initiator that is bound to the surface of the particle.

20 16. A method according to claim 15, wherein the azoinitiator is bound to the surface of the particle by a two point attachment.

17. A method according to claim 16, wherein the azoinitiator is the reaction product of glycidoxypentyl-  
25 trimethoxysilane (GPS) and azo-bis(cyanopentanoic acid) (ACPA).

18. A method according to claim 14, wherein the initiator is an azo-bis-amidine initiator that is adsorbed to the surface of the support and is insoluble  
30 in the polymerisation medium.

19. A method according to claim 18, wherein the initiator is 2,2'-azo-bis(2-amidinopropane) or 2,2'-azobis(N,N'-dimethyleneisobutyramidine).

20. A method according to claim 12, wherein the  
35 polymerisation is confined to the surface of the support by subjecting the composition to microwave irradiation

which selectively heats the support and thereby initiates a polymerisation reaction at the surface of the support.

21. A method according to any one of claims 12-20, wherein the polymerisation on the support is repeated at least once with a different composition to obtain at least one further layer of a molecularly imprinted polymer; a layer of different polarity; or a layer of other functional properties.

22. Use of a molecularly imprinted polymer according to any one of claims 1-11, or prepared according to any one of claims 12-21, in chromatography, for separations, in chemical sensors, in molecular recognition as stationary phase in capillaries, in selective sample enrichment or in catalysis.

23. Azoinitiator as a means of carrying out the method of any one of claims 12-21, characterised in that it is the reaction product of glycidoxypolypropyltrimethoxysilane (GPS) and azo-bis-(cyanopentanoic acid) (ACPA).

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number  
WO 01/19886 A1(51) International Patent Classification<sup>7</sup>: C08F 291/00,  
285/00, 292/00

(21) International Application Number: PCT/SE00/01776

(22) International Filing Date:  
14 September 2000 (14.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
9903387-0 17 September 1999 (17.09.1999) SE  
0000294-9 28 January 2000 (28.01.2000) SE(71) Applicant (for all designated States except US): MIP  
TECHNOLOGIES AB [SE/SE]; Research Park Ideon,  
S-223 70 Lund (SE).

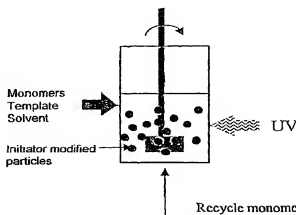
(72) Inventors; and

(75) Inventors/Applicants (for US only): SELLERGREN,  
Börje [SE/DE]; University of Mainz, Department of  
Inorganic Chemistry and Analytical Chemistry, Duesberg-  
weg 10-14, 55099 Mainz (DE). SULITZKY, Claudia  
[DE/DE]; University of Mainz, Department of InorganicChemistry and Analytical Chemistry, Duesbergweg 10-14,  
55099 Mainz (DE). RÜCKERT, Bärbel [DE/DE]; Uni-  
versity of Mainz, Department of Inorganic Chemistry and  
Analytical Chemistry, Duesbergweg 10-14, 55099 Mainz  
(DE).(74) Agent: AWAPATENT AB; Box 5117, S-200 71 Malmö  
(SE).(81) Designated States (*national*): AE, AG, AL, AM, AT, AT  
(utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA,  
CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility  
model), DK, DK (utility model), DM, DZ, EE, EE (utility  
model), ES, FI, FI (utility model), GB, GD, GE, GH, GM,  
HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility  
model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG,  
MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD,  
SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT,  
TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,  
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

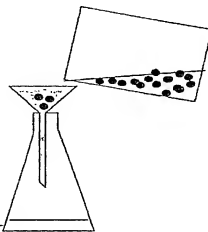
[Continued on next page]

(54) Title: NEW MOLECULARLY IMPRINTED POLYMERS GRAFTED ON SOLID SUPPORTS

## A. Graft polymerization

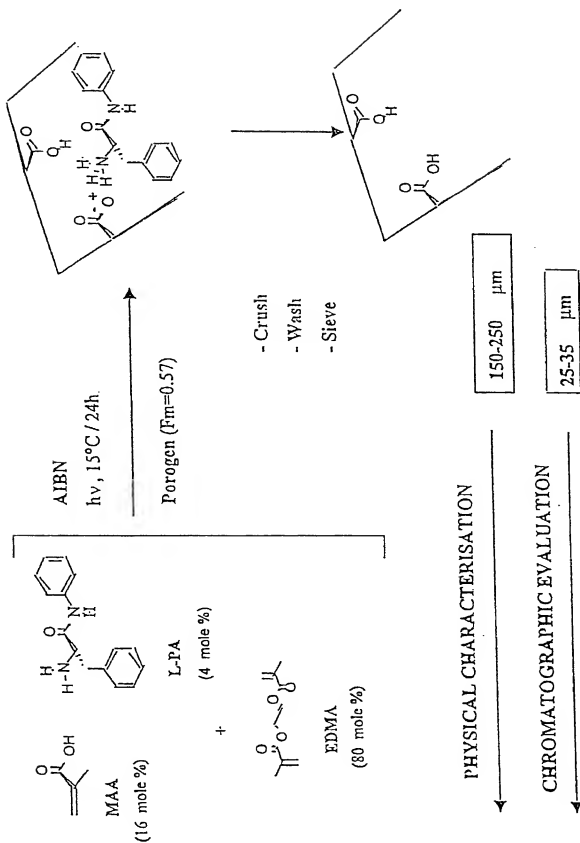


## B. Filter, wash, and dry the particles



(57) Abstract: The invention refers to a molecularly imprinted polymer, a method of preparing a molecularly imprinted polymer material, and the use thereof. According to the invention a support and a composition comprising at least one monomer, and a template, in a polymerisation medium is polymerised with a free radical initiator, whereafter the template is removed from the molecularly imprinted polymer obtained. The polymerisation is confined to the surface of the support, preferably by confining the free radical initiator to the support by bonding or adsorption. The molecularly imprinted polymer may be used in chromatography, for separations, in chemical sensors, in molecular recognition as stationary phase in capillaries, in selective sample enrichment or in catalysis.

Figure 1





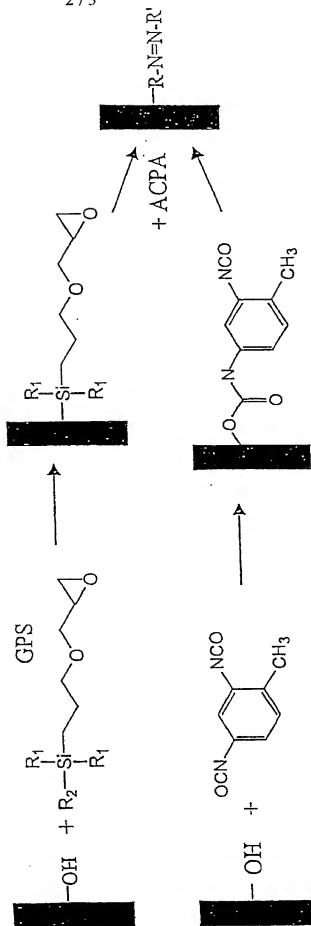
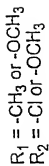
CN(C)CCC(=O)O/C=C/C(C)(C)CC(O)C#N  
= ACPA

Figure 3

A. Graft polymerization

B. Filter, wash, and dry  
the particles

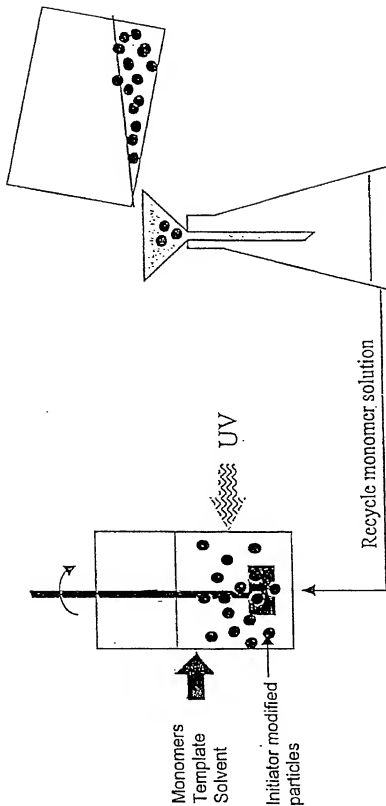
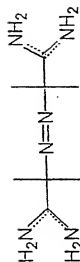
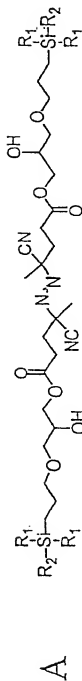


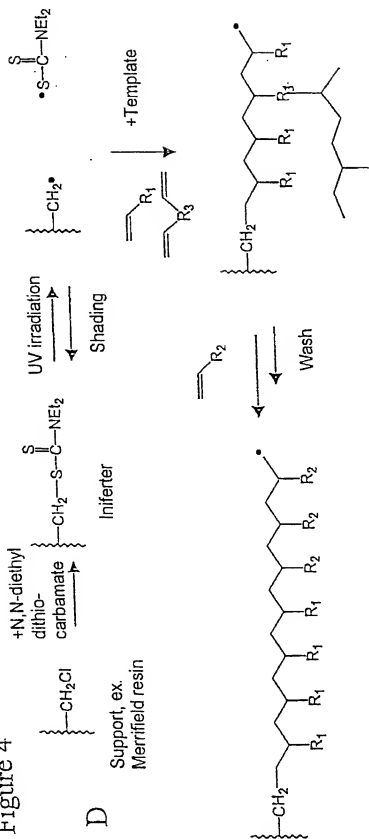
Figure 4



Microwave

C

Figure 4



**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and International (PCT) Applications)**Attorney's Docket  
No. 003300-908

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (IF ONLY ONE NAME IS LISTED BELOW) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (IF PLURAL NAMES ARE LISTED BELOW) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED:

The specification of which (check only one item below):

- ☐ is attached hereto.
- ☐ was filed as United States Patent Application Number \_\_\_\_\_  
on \_\_\_\_\_  
and was amended on \_\_\_\_\_ (if applicable).
- ☐ was filed as International (PCT) Application Number \_\_\_\_\_  
on \_\_\_\_\_  
and was amended on \_\_\_\_\_ (if applicable).

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I ACKNOWLEDGE THE DUTY TO DISCLOSE TO THE U.S. PATENT AND TRADEMARK OFFICE ALL INFORMATION KNOWN TO ME TO BE MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, Sec. 1.56 (as amended effective March 16, 1992);

I do not know and do not believe the said invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application; that said invention was not in public use or on sale in the United States of America more than one year prior to said application; that said invention has not been patented or made the subject of an inventor's certificate issued before the date of said application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than six months prior to said application;

I hereby claim foreign priority benefits under Title 35, United States Code, §§ 119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any International (PCT) Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International (PCT) Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

**PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:**

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Sweden	9903387-0	17 September 1999	X Yes <input type="checkbox"/> No
Sweden	0000294-9	28 January 2000	X Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

(APPLICATION NUMBER)

(FILING DATE)

(APPLICATION NUMBER)

(FILING DATE)

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D)**  
(Includes Reference to Provisional and International (PCT) Applications)Attorney's Docket  
No. 003300-908

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or International (PCT) Application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations § 1.56, which became available between the filing date of the prior application(s) and the national or international filing date of this application:

PRIOR U.S. APPLICATIONS OR INTERNATIONAL (PCT) APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. § 120:

U.S. APPLICATIONS		STATUS (check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED
		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PCT APPLICATIONS DESIGNATING THE U.S.				
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)		
SE00/01776	14 September 2000			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the U.S. Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	Eric H. Wesblatt	30,505	Bruce T. Wieder	33,815
Robert S. Swecker	19,885	James W. Peterson	26,057	Todd R. Walters	34,040
Platon N. Mandros	22,124	Teresa Stanek Rea	30,427	Ronni S. Jillions	31,979
Benton S. Duffett, Jr.	22,030	Robert E. Krebs	25,885	Harold R. Brown III	36,341
Norman H. Stepano	22,716	William C. Rowland	30,888	Allen R. Baum	36,086
Ronald L. Gudzicki	24,970	T. Gene Dillahunty	25,423	Steven M. duBois	35,023
Frederick G. Mischaud, Jr.	26,003	Patrick C. Keane	32,858	Brian P. O'Shaughnessy	32,747
Alan E. Kopecki	25,813	B. Jefferson Boggs, Jr.	32,344	Kenneth B. Leffler	36,075
Regis E. Slutter	26,999	William H. Benz	25,952	Fred W. Hathaway	32,236
Samuel C. Miller, III	27,360	Peter K. Skiff	31,917	Wendi L. Weinstein	34,456
Robert G. Mukai	28,531	Richard J. McGrath	29,195	Mary Ann Dillahunty	34,576
George A. Hovanec, Jr.	28,223	Matthew L. Schneider	32,814		
James A. LaBarre	28,632	Michael G. Savage	32,596		
E. Joseph Gess	28,510	Gerald F. Swiss	30,113		
R. Danny Huntingdon	27,903	Charles F. Wieland III	33,096		



21839

and: \_\_\_\_\_

Address all correspondence to:

BURNS, DOANE, SWECKER & MATHIS, L.L.P.  
P.O. Box 1404  
Alexandria, Virginia 22313-1404




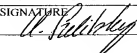
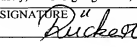
21839

Address all telephone calls to: \_\_\_\_\_ at (703) 836-6620.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D)**  
 (Includes Reference to Provisional and International (PCT) Applications)

 Attorney's Docket  
 No. 003300-908

FULL NAME OF SOLE OR FIRST INVENTOR <u>Birge SELLERGRÉN</u>		SIGNATURE 	DATE <u>16 January 2002</u>
RESIDENCE (CITY & STATE/COUNTRY) <u>Mainz, Germany DEX</u>		CITIZENSHIP <u>Swedish</u>	
POST OFFICE ADDRESS (HOME ADDRESS) <u>University of Mainz, Department of Inorganic Chemistry and Analytical Chemistry, Duesbergweg 10-14, 55099 MAINZ, GERMANY</u>			
FULL NAME OF SECOND JOINT INVENTOR, IF ANY <u>Claudia SULITZKY</u>		SIGNATURE 	DATE <u>16 January 2002</u>
RESIDENCE (CITY & STATE/COUNTRY) <u>Mainz, Germany DEX</u>		CITIZENSHIP <u>German</u>	
POST OFFICE ADDRESS (HOME ADDRESS) <u>University of Mainz, Department of Inorganic Chemistry and Analytical Chemistry, Duesbergweg 10-14, 55099 MAINZ, GERMANY</u>			
FULL NAME OF THIRD JOINT INVENTOR, IF ANY <u>Barbel RÜCKERT</u>		SIGNATURE 	DATE <u>16 January 2002</u>
RESIDENCE (CITY & STATE/COUNTRY) <u>Mainz, Germany DEX</u>		CITIZENSHIP <u>German</u>	
POST OFFICE ADDRESS (HOME ADDRESS) <u>University of Mainz, Department of Inorganic Chemistry and Analytical Chemistry, Duesbergweg 10-14, 55099 MAINZ, GERMANY</u>			
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE (CITY & STATE/COUNTRY)		CITIZENSHIP	
POST OFFICE ADDRESS (HOME ADDRESS)			
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE (CITY & STATE/COUNTRY)		CITIZENSHIP	
POST OFFICE ADDRESS (HOME ADDRESS)			
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE (CITY & STATE/COUNTRY)		CITIZENSHIP	
POST OFFICE ADDRESS (HOME ADDRESS)			
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE (CITY & STATE/COUNTRY)		CITIZENSHIP	
POST OFFICE ADDRESS (HOME ADDRESS)			
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE (CITY & STATE/COUNTRY)		CITIZENSHIP	
POST OFFICE ADDRESS (HOME ADDRESS)			
FULL NAME OF NINTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE (CITY & STATE/COUNTRY)		CITIZENSHIP	
POST OFFICE ADDRESS (HOME ADDRESS)			
FULL NAME OF TENTH JOINT INVENTOR, IF ANY		SIGNATURE	DATE
RESIDENCE (CITY & STATE/COUNTRY)		CITIZENSHIP	
POST OFFICE ADDRESS (HOME ADDRESS)			